

Aberrant mRNAs with extended 3' UTRs are substrates for rapid degradation by mRNA surveillance

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ABSTRACT

The mRNA surveillance system is known to rapidly degrade aberrant mRNAs that contain premature termination codons in a process referred to as nonsense-mediated decay. A second class of aberrant mRNAs are those wherein the 3' UTR is abnormally extended due to a mutation in the polyadenylation site. We provide several observations that these abnormally 3'-extended mRNAs are degraded by the same machinery that degrades mRNAs with premature nonsense codons. First, the decay of the 3'-extended mRNAs is dependent on the same decapping enzyme and 5'-to-3' exonuclease. Second, the decay is also dependent on the proteins encoded by the UPF1, UPF2, and UPF3 genes, which are known to be specifically required for the rapid decay of mRNAs containing nonsense codons. Third, the ability of an extended 3' UTR to trigger decay is prevented by stabilizing sequences within the PGK1 coding region that are known to protect mRNAs from the rapid decay induced by premature nonsense codons. These results indicate that the mRNA surveillance system plays a role in degrading abnormally extended 3' UTRs. Based on these results, we propose a model in which the mRNA surveillance machinery degrades aberrant mRNAs due to the absence of the proper spatial arrangement of the translation-termination codon with respect to the 3' UTR element as defined by the utilization of a polyadenylation site.

Keywords: 3' UTR; mRNA decay; mRNA turnover; nonsense-mediated decay; yeast

INTRODUCTION

An important issue within cells is the accurate production and translation of mRNA molecules. In the last few years it has become clear that a specialized pathway of mRNA degradation exists to degrade transcripts that contain premature translation-termination codons. This process has been referred to as nonsense-mediated decay (Peltz et al., 1993) or mRNA surveillance (Pulak & Anderson, 1993). The biological role of this process is potentially larger than simply degrading mRNAs containing nonsense mutations as the process of mRNA surveillance degrades unspliced pre-mRNAs that reach the cytoplasm (He et al., 1993). Moreover, the products of alternative splicing can be subjected to differential decay by mRNA surveillance (Morrison et al., 1997). Thus, an understanding of the diversity of substrates that are degraded by the mRNA surveillance process

will help to illuminate the biological role of mRNA surveillance in eukaryotic cells.

Another class of potential substrates for mRNA surveillance are aberrant mRNAs that are altered in the position of 3' end formation and polyadenylation. This type of transcript has been observed to occur both in yeast (Zaret & Sherman, 1984) and in nematodes (Pulak & Anderson, 1993) when the chromosome contains a mutation affecting the normal polyadenylation signals. In these cases, cryptic polyadenylation sites further downstream are used for 3' end formation. This leads to the production of mRNAs with aberrantly long 3' untranslated regions (UTR) (Zaret & Sherman, 1984; Pulak & Anderson, 1993). Two observations suggest that these transcripts might be degraded by the mRNA surveillance pathway. First, the aberrant 3'-extended transcripts are present at low steady-state levels, suggesting that they are unstable (Zaret & Sherman, 1984; Pulak & Anderson, 1993). Second, *trans*-acting mutations in *Caenorhabditis elegans* that increase the levels of such an aberrant 3'-extended transcript from the unc-54 gene also increase the low levels of mRNAs

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that contain early nonsense codons (Pulak & Anderson, 1993).

To determine if aberrant 3'-extended transcripts are actually degraded by the mRNA surveillance process, we took advantage of the understanding of mRNA turnover mechanisms, including mRNA surveillance, that has occurred in yeast. We demonstrate by several criteria that the degradation of 3'-extended transcripts is the same as the degradation of mRNAs that contain early nonsense codons. The recognition that 3'-extended transcripts are degraded by the same machinery that degrades mRNAs with early nonsense codons suggests a possible model for how aberrant mRNAs are targeted for degradation.

RESULTS

An extended 3' UTR is a transferable mRNA destabilizing sequence

A well-characterized example of a yeast mRNA that is aberrant due to a 3' extension is the analysis of the *cyc1-512* mutation. This mutation in the *CYC1* gene deletes the sequences required for the proper formation of the 3' end. As a result, a low level of 3'-extended transcripts with end points at several cryptic polyadenylation sites 3' of the normal site are produced (Zaret & Sherman, 1984). This suggests that transcripts with abnormal 3' ends are recognized as aberrant and degraded as such within the cell.

To determine if the extended 3' ends were sufficient to promote mRNA degradation, we asked if transferring the extended 3' ends onto a reporter mRNA would lead to its destabilization. To do this experiment, we replaced the 3' end of a gene encoding a *CUP1* reporter mRNA with ~1.5 kb from the 3' end of the wild-type *CYC1* gene or the same region containing the *cyc1-512* allele (see Materials and Methods). This 1.5-kb region was chosen because it contained several of the cryptic polyadenylation sites previously described (Zaret & Sherman, 1984).

Analysis of steady-state levels of the reporter mRNAs containing the wild-type *CYC1* 3' sequences identified a discrete band of approximately 500 nt, which is as expected for utilization of the normal *CYC1* polyadenylation site (Fig. 1A, lane 2). In contrast, the construct containing the *cyc1-512* sequences produced a variety of transcripts that, based on their sizes, had 3' ends positioned either at the same sites as originally described (Zaret & Sherman, 1984) to beyond the *CYC1* sequences and into the plasmid itself (Fig. 1A, lane 1). This result indicated that transferring the 3' UTR containing the *cyc1-512* mutation to a reporter mRNA would still give rise to 3'-extended transcripts.

We also constructed a gene that included the 3' end formation site from the *PGK1* gene positioned downstream of the 1.5 kb of inserted *CYC1* sequences (see

Materials and Methods). The *PGK1* sequences provided a bona fide 3' end formation site to provide, we hope, a discrete 3' end on any transcripts wherein the upstream cryptic 3' end formation sites were not utilized. In this case, the majority of *cyc1-512* transcripts terminated at the *PGK1* 3' end formation site located ~1.5 kb further downstream. (Fig. 1A, lane 3). This suggests that the 3' end formation sites within the *CYC1* sequences are likely to be inefficient sites for 3' end formation that may only be used in the absence of a true polyadenylation signal. Because the population of *cyc1-512*-containing transcripts were still 3' extended, but more discrete when the *PGK1* polyadenylation site was included, we focused our mRNA analysis on this class of transcripts (see below), although similar results have been observed with the mRNAs terminating at the cryptic polyadenylation sites (data not shown).

To determine if the extended 3' ends produced due to the *cyc1-512* mutations would alter mRNA stability, we directly measured the decay rate of the transcripts following repression of the reporter by the addition of glucose (see Materials and Methods). The control transcript containing the wild-type *CYC1* 3' formation sequences exhibited a decay rate of approximately 6 min in wild-type cells (Fig. 2B, top panel). This decay rate is roughly equivalent to the decay rates of the *CUP1* mRNA ($t_{1/2}$ = 10 min) and the *CYC1* mRNA ($t_{1/2}$ = 7 min).

In contrast, examination of the decay rate of the 3'-extended transcript containing the *cyc1-512* 3' sequences (Fig. 3A) in wild-type cells showed an accelerated decay rate ($t_{1/2}$ = 3 min, Fig. 3B, top panel) compared to the shorter transcript with the proper *CYC1* 3' end ($t_{1/2}$ = 6 min; Fig. 2B). This result demonstrated that the extended 3' ends were sufficient to accelerate decay of a reporter mRNA. In principle, extended 3' ends could promote mRNA decay because cryptic polyadenylation sites lead to the altered assembly of 3' UTR binding proteins, thereby leading to decay. However, as these 3'-extended transcripts utilize the normal *PGK1* polyadenylation site, these results indicated that the rapid decay of such 3'-extended transcripts is not caused by the use of inefficient, cryptic polyadenylation sites per se, and instead is likely to be a consequence of the other altered features of the transcript (see below).

Aberrant 3'-extended mRNAs are decapped and degraded 5' to 3' due to mRNA surveillance

The above results indicated that improperly extended 3' UTRs can destabilize a transcript. As discussed in the introduction, the phenotypes of *smg* mutants in *C. elegans* suggested that these 3' extensions might direct the transcript to the mRNA surveillance pathway known to degrade mRNAs containing early nonsense

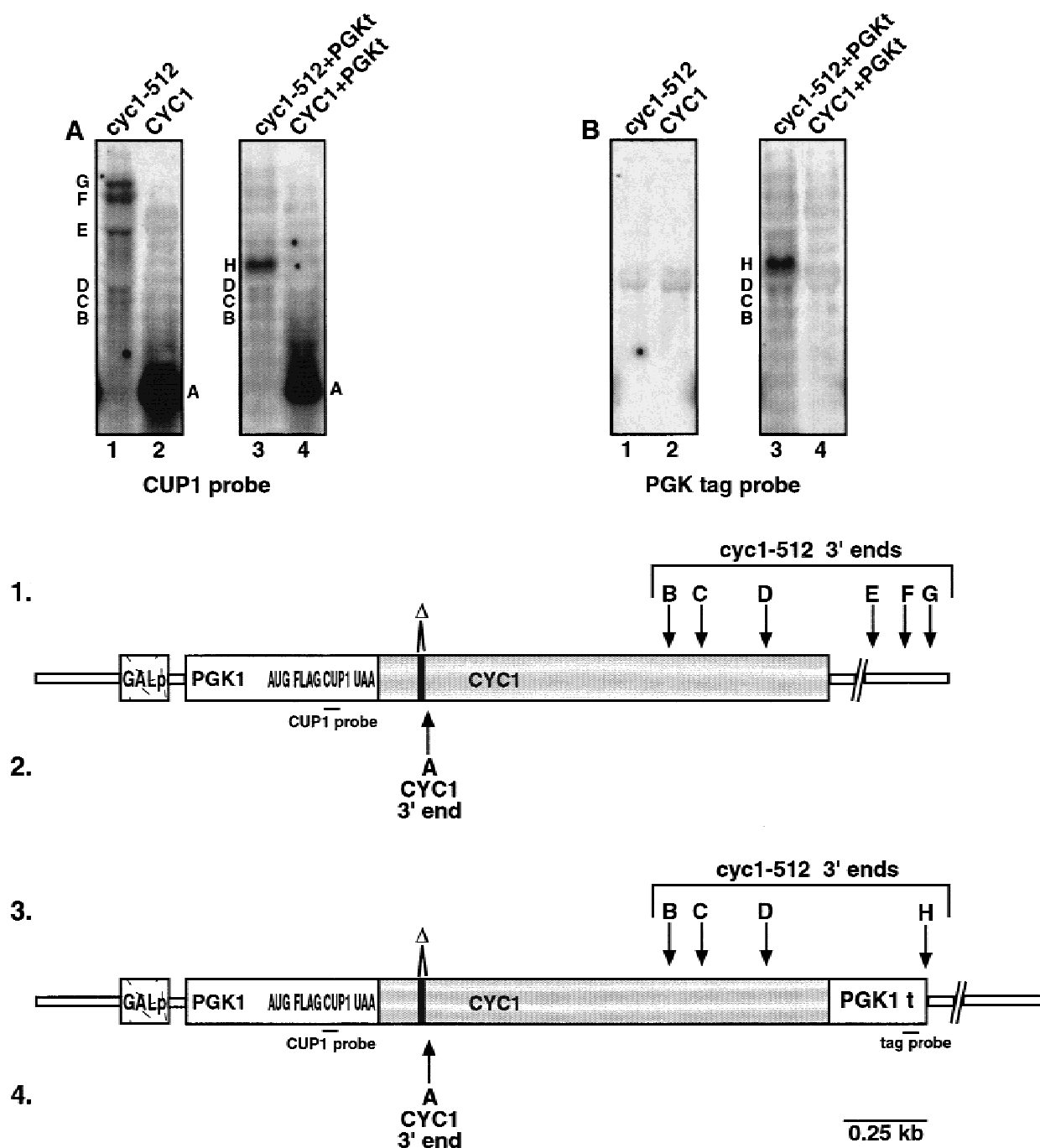


FIGURE 1. Transcripts produced from *CYC1* and *cyc1-512* containing constructs. **A** and **B** show agarose northern blots with 10 μ g of total mRNA from wild-type strains showing the mRNA species present in four different reporter constructs containing either wild-type *CYC1* or mutant *cyc1-512* sequences in each lane. Above each lane is the corresponding construct name. The presence of a 3' PGK1 terminating sequence is designated as PGKt. Next to each band is a letter that refers to the mRNA species of the approximate sizes given below. Lanes are numbered below for each panel that correspond to the mRNA species depicted in the cartoons below. **A:** Northern blots are probed for the CUP1 mRNA sequences present in all of these constructs. **B:** Identical northern blots as in **A** but probed for the 3' PGK tag sequence. Cartoons below the northern blots show the plasmid constructs from which each mRNA was made. The numbers on the left refer to the northern lanes above in **A** and **B**. The skinny open rectangles represent vector sequences; PGK1 sequences are in large open rectangles; inserted flag and CUP1 sequences are also shown; *CYC1* or *cyc1-512* sequences are shown as a single gray box; the polyadenylation signal sequences, which are deleted in the *cyc1-512* constructs, are shown as a black box; the location of the two probes used are indicated as lines under each construct. Letters designating the 3' ends of the mRNA species seen in the upper northern blots are marked with arrows. Species A-D match previously reported sizes (Zaret & Sherman, 1984) of mRNA species produced from the *CYC1* and *cyc1-512* sequences. Approximate sizes in bases of these mRNA species are as follows: A: 530; B: 1250; C: 1350; D: 1550; E: 3200; F: 3500; G: 4500; H: 2200.

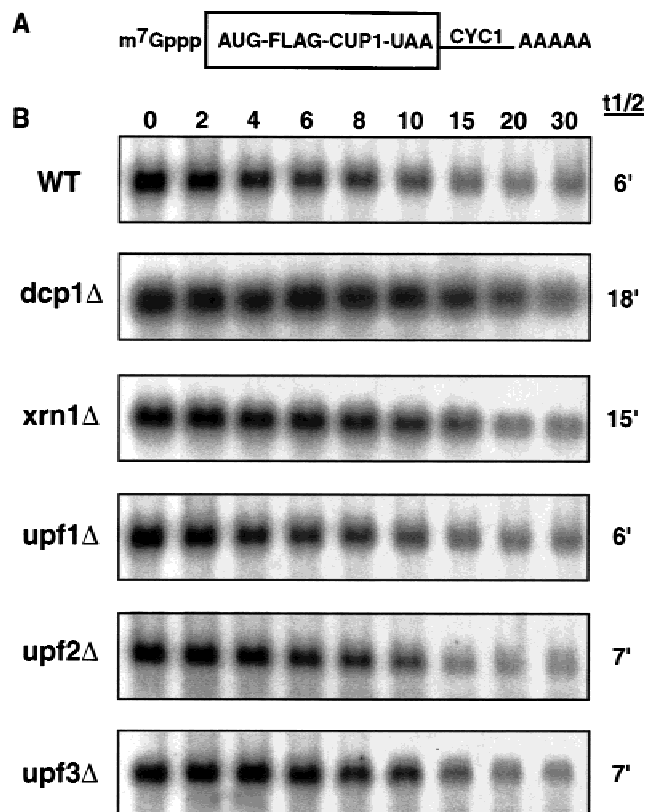


FIGURE 2. Deletion strains containing the wild-type CYC1 3' end and their half-lives. **A:** A cartoon showing the mRNA species made in each strain. **B:** Half-life experiments for the mRNA depicted in **A** in each of six different isogenic strains. Strain names are given on the left of each northern panel. Numbers above the panels are the time points in minutes after transcription repression. To the right of each panel are the respective half-lives for each strain in minutes determined from at least two experiments with an error of less than ± 0.5 min. Northern blots are probed with an oligonucleotide (oRP674) complementary to the very 5' portion of the CYC1 sequence.

codons (Pulak & Anderson, 1993). To test this hypothesis, we examined the effects of *trans*-acting mutations known to alter the mRNA surveillance pathway to determine if they affect the decay of the 3'-extended mRNAs.

Transcripts that contain early nonsense codons are degraded by a deadenylation-independent decapping reaction, which leads to 5'-to-3' exonuclease degradation. The enzymes responsible for these reactions are the Dcp1p decapping enzyme (Beelman et al., 1996; LaGrandeur & Parker, 1998) and the Xrn1p 5'-to-3' ribonuclease (Muhlrud & Parker, 1994; Hagan et al., 1995). As shown in Figure 3B, the 3'-extended transcripts were stabilized in both *dcp1Δ* and *xrn1Δ* strains. These observations argued that decay of these aberrant mRNAs proceeded by decapping and then degrading in a 5'-to-3' direction.

The DCP1 and XRN1 gene products are also required for the decapping and 5'-to-3' degradation of normal mRNAs that occurs following deadenylation

(Fig. 2; Decker & Parker, 1993; Hsu & Stevens, 1993; Muhlrud et al., 1994; Beelman et al., 1996). In contrast, the degradation of transcripts with early nonsense codons by the mRNA surveillance pathway is inhibited in strains lacking the UPF1, UPF2, or UPF3 genes without an effect on normal mRNA degradation (Leeds et al., 1991; Lee & Culbertson, 1995; Cui et al., 1996; He et al., 1996). Therefore, to determine if the 3'-extended mRNAs were specifically being degraded by the mRNA surveillance pathway, we determined if deletion of the UPF1, UPF2, or UPF3 genes affected their degradation. An important result was that the 3'-extended mRNAs were stabilized in strains lacking Upf1p, Upf2p, or Upf3p proteins (Fig. 3B). In contrast, the decay rate of the fusion mRNA with the normal CYC1 3' UTR was not significantly affected by the deletion of any of the Upf genes (Fig. 2B). The stabilization of the improperly 3'-extended transcripts in the *upf1Δ*, *upf2Δ*, and *upf3Δ* strains indicated that these mRNAs were being recognized as aberrant by the same

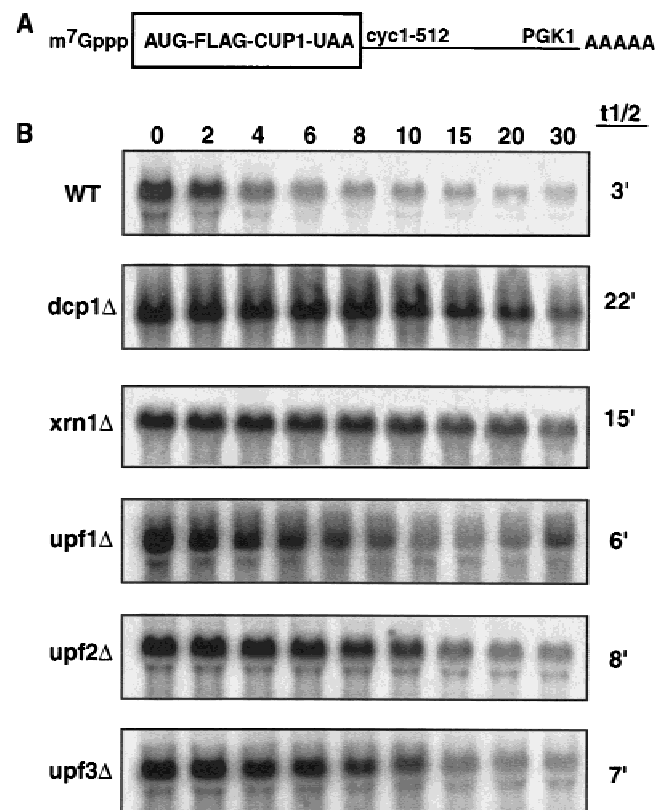


FIGURE 3. Comparison of the half-lives of *cyc1-512*-containing mRNAs in wild-type and several deletion strains. **A:** Depiction of the predominant mRNA species harboring the *cyc1-512* mutation made in the experiments shown in **B**. **B:** mRNA half-lives of the *cyc1-512*-containing mRNA in the six strains listed at the left of each northern panel. The time in minutes after repression of transcription is shown above each column in the northern panels. The corresponding half-life measurements for each strain are shown to the right. Each value is from at least two experiments with an error of less than ± 0.5 min. These northern blots are probed with a complementary CYC1 oligonucleotide.

machinery that recognizes mRNAs with early non-sense codons.

A simple phenotypic assay for mRNA surveillance

Useful tools for genetic analysis are phenotypes that reflect underlying biological processes. Previous genetic assays in yeast for the process of mRNA surveillance are limited in that they have relied on the readthrough of nonsense codons and/or frameshifting sites and therefore require multiple effects to observe a phenotypic change (Leeds et al., 1991; Weng et al., 1996). The reporter mRNA we constructed expressed the CUP1 polypeptide, which allows growth on media containing Cu^{2+} , upstream of either the aberrant or normal CYC1 3' UTRs. Thus, we predicted that strains expressing the aberrant transcripts would show a reduced ability to grow on Cu^{2+} -containing media and that this Cu^{2+} sensitivity would be suppressed by inactivation of the mRNA surveillance pathway. As shown in Figure 4, strains expressing the 3'-extended transcripts due to the *cyc1-512* lesion were significantly more copper sensitive than strains expressing the construct with the normal CYC1 3' UTR. Moreover, the ability of strains expressing the 3'-extended mRNAs to grow on Cu^{2+} -containing media was restored in strains deleted for the *upf1* gene. This simple phenotypic assay for the mRNA surveillance process should be useful in the genetic analysis of the mRNA surveillance pathway.

The extended 3' destabilizing sequences are inactivated by sequences that block the ability of a nonsense codon to stimulate degradation

The observation that the decay of the 3'-extended transcripts was reduced and the transcripts were stabilized in the *upf1*, *upf2*, and *upf3* deletion strains (Fig. 3B) suggests that these transcripts are distinguished from

normal mRNAs by the same mechanism that differentiates mRNAs with early nonsense codons from normal mRNAs. In the case of transcripts with early nonsense codons, additional sequences 3' of the termination codon, referred to as downstream elements or DSEs (Peltz et al., 1993), are required to promote mRNA degradation. Interestingly, the ability of these DSEs to promote mRNA turnover is prevented by translation of the complete PGK1 coding region, which had therefore been inferred to contain a stabilizing sequence (Peltz et al., 1993). To obtain additional evidence that the improperly extended 3' UTRs were leading to degradation in a manner similar to premature nonsense codons, we determined if the ability of the 3'-extended ends to promote decay was similarly inhibited by translation of the complete PGK1 coding region.

To do this experiment, we replaced the 3' UTR of the full-length PGK1 mRNA with either the wild-type or *cyc1-512* 3' ends, again followed by the PGK1 3' end formation signal, and examined the degradation of the resulting mRNAs (Fig. 5A). As expected, the wild-type CYC1 sequences directed 3' end formation at the position seen in the CYC1 mRNA in both wild-type and *upf1* Δ strains (Fig. 5, right panels). Half-lives of the CYC1-containing mRNAs were nearly identical in these strains ($t_{1/2} = 10$ min and $t_{1/2} = 9$ min, respectively). This indicated that the CYC1 3' UTR could destabilize the PGK1 transcript, although that destabilization was independent of Upf1p and therefore unrelated to mRNA surveillance. Similar results have been seen with 3' UTRs from other unstable mRNAs and are consistent with specific 3' UTR regions having features that accelerate the normal mechanism of mRNA decay (e.g., see LaGrandeur & Parker, 1999).

When the *cyc1-512* sequences were present after the full PGK1 coding region, they led to the formation of 3'-extended transcripts with the majority of 3' ends being formed at the downstream PGK1 terminator (data not shown). The half-lives of these extended transcripts were also similar in wild-type ($t_{1/2} = 9$ min) and *upf1* Δ strains ($t_{1/2} = 11$ min) (Fig. 5A). An important comparison is that the full-length PGK1 transcripts with the wild-type CYC1 3' end ($t_{1/2} = 10$ min) and the full-length PGK1 transcripts with the extended 3' ends had essentially the same decay rate ($t_{1/2} = 9$ min) either in wild-type or *upf1* Δ strains (Fig. 5A). These observations indicated that the improper 3'-extended region was not able to promote mRNA surveillance when placed 3' of the PGK1 coding region. This suggests that the improper 3' UTR extensions behave similarly to a premature termination codon in response to the stabilizing effect of the PGK1 coding region.

To verify that the stabilization of transcripts containing extended 3' UTRs was due to the same effect of the PGK1 coding region that prevents nonsense codons from triggering decay, we constructed a pair of transcripts wherein the stabilizing region of the PGK1

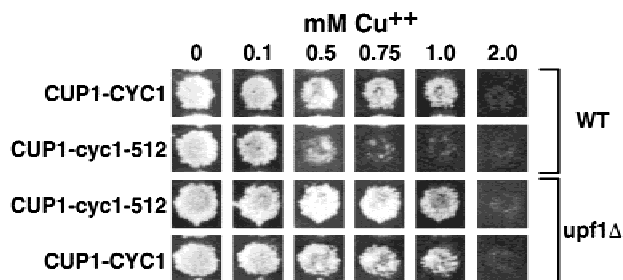


FIGURE 4. Phenotypes of wild-type and *upf1* Δ strains containing CYC1 or *cyc1-512* reporter constructs. Strains are given to the right of the rows and the constructs contained in each strain are listed on the left of each row. The copper concentrations are given above each column. Strains were grown for 3 days on selective minimal Galactose plates before being photographed.

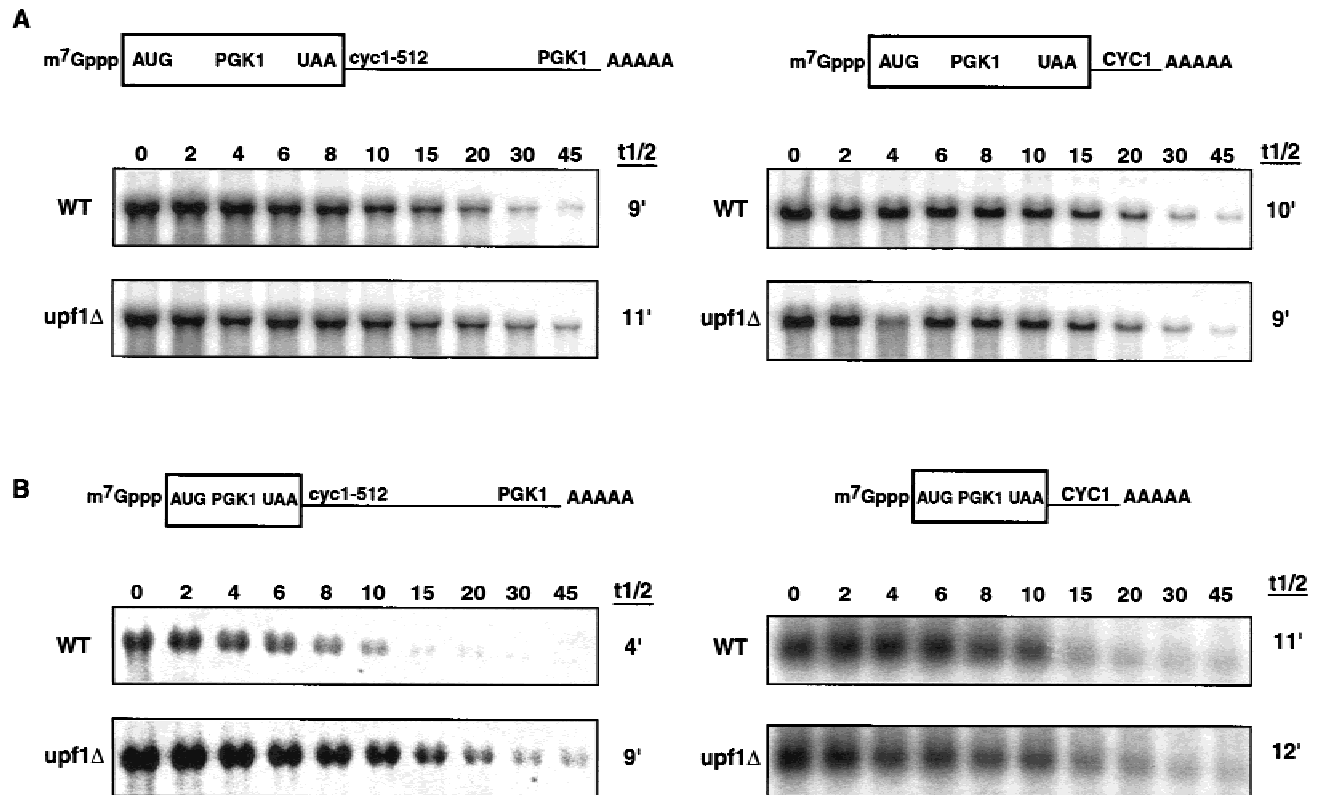


FIGURE 5. Half-lives comparing the insertion of wild type (WT), *CYC1*, and *cyc1-512* sequences behind the complete coding region or a shortened nonsense-containing region of the *PGK1* mRNA. **A:** A representative cartoon of the mRNA produced from each construct containing the complete coding region of *PGK1* is shown above each side of the northern panels. **B:** A cartoon of the mRNAs made containing only a short portion of the *PGK1* coding region (ending 103 bases into the mRNA) followed by either the *CYC1* or *cyc1-512* sequences. **A,B:** Strain names are to the left of each northern and half-life values are at the right of each panel. Half-life values given are from two experiments with an error of less than ± 1.0 min. Minutes after transcription repression are shown directly above the upper northern in each series. Blots are probed with an oligo complementary to *CYC1*.

coding region (Peltz et al., 1993) was removed. Consistent with the extended 3' UTR acting like nonsense codons, these transcripts were now destabilized in the wild-type strain by the extended *cyc1-512* ends (Fig. 5B). Additionally these same mRNAs were stabilized in the *upf1Δ* strains (Fig. 5B), further demonstrating their true nonsense character.

DISCUSSION

3'-extended transcripts are substrates for mRNA surveillance

Several observations now indicate that aberrant 3'-extended mRNAs are substrates for degradation by the mRNA surveillance system. First, such 3'-extended transcripts are present at low steady-state levels (Zaret & Sherman, 1984; Pulak & Anderson, 1993), and show accelerated rates of mRNA degradation as compared to control mRNAs (Figs. 2 and 3). Second, the rapid decay of these mRNAs was dependent upon the Dcp1p decapping enzyme and the Xrn1p 5'-to-3' exonucle-

ase, which are also required for the rapid decay of mRNAs with premature termination codons (Fig. 3). Third, the rapid decay of the 3'-extended transcripts was inhibited in strains lacking either Upf1p, Upf2p, or Upf3p (Fig. 3). In addition, recent results indicate that a suppressor of the *cyc1-512* mutation, termed *sut1*, is identical to the *UPF1* gene (Fred Sherman, pers. comm.). Thus, the mRNA surveillance system plays a role in degrading the aberrant mRNAs that arise from chromosomal mutations affecting polyadenylation sites.

One implication of this work is that 3'-extended transcripts that arise due to the failure to recognize and utilize a site of 3' end processing might be recognized and degraded by the mRNA surveillance pathway. This raises the possibility that the fidelity of 3' end formation may not be as high as thought and that errors in this process may be largely undetectable because of rapid degradation by the mRNA surveillance mechanism. Degradation of such aberrantly extended 3' mRNAs could be biologically important in that it would prevent the translation of aberrant mRNAs lacking the proper 3' UTR regulatory signals, which in many cases are

known to function in specifying transcript localization, translation, and turnover rate (for review, see Decker & Parker, 1995).

Distinguishing between normal and extended 3' UTRs

How are the normal and extended 3' transcripts distinguished in yeast by the mRNA surveillance mechanism? The data now indicate that the rapid degradation of aberrant 3'-extended mRNAs requires the same machinery that distinguishes mRNAs with premature termination codons from normal mRNAs. This fact implies that mRNAs with premature termination codons and extended 3' UTRs are distinguished from normal mRNAs by a similar mechanism. Both types of aberrant transcripts share two key differences from normal mRNAs. First, in both cases additional sequences are found within the 3' UTR sequences that are not normally found in that position. In the case of 3'-extended mRNAs these sequences arise from the downstream nucleotides that are not normally transcribed. In the case of premature termination codons, these additional 3' UTR sequences arise from the portion of the coding region that is now 3' of the premature termination codon. The second difference is that in both cases, the translation-termination codon is no longer located near the site of polyadenylation. In other words, the proper arrangement of the translation-termination codon and the site of polyadenylation is now altered.

These differences suggest two views of the possible manner in which aberrant mRNAs are distinguished from normal mRNAs and targeted for mRNA degradation. In one hypothesis, the additional sequences 3' of the termination codon in the aberrant transcripts would be recognized by the mRNA surveillance machinery and this recognition would then trigger mRNA degradation (see Nagy & Maquat, 1998; Czapinski et al., 1999; Hentze & Kulozik, 1999).

An alternative hypothesis is that there is an alteration of the proper spatial arrangement between the translation-termination codon and elements within the normal 3' UTR that are normally required for stabilization of the mRNA. An important aspect of this model is that the formation of the assembly of proteins on the 3' UTR, which are hypothesized to interact with events at the translation-termination codon, would occur during the recognition and use of the site of 3' end formation and polyadenylation. In this view, the presence of a premature translation-termination codon would disrupt the proper interactions by moving the site of translation termination too far 5'. In contrast, the alteration of the site of 3' end formation and polyadenylation would alter the interaction by moving the site of 3' end formation, and concurrent assembly of 3' UTR-bound proteins too far 3'. Consistent with a need for interactions between the assembly of proteins on the 3' UTR that play

a role in polyadenylation and the translation-termination process, yeast 3' UTRs are generally relatively short (~100 nt) and homogeneous in length (Graber et al., 1999).

The proposal that there is a specific coupling between the process of 3' end formation and the cytoplasmic fate of an mRNA is supported by several observations in the literature. First, multiple proteins are involved in both the formation of the 3' poly(A) tail and the cytoplasmic stability of the mRNA. For example, the poly(A) binding protein (Pab1p) is required for polyadenylation of pre-mRNAs (Amrani et al., 1997) and is required for the poly(A) to serve as an inhibitor of mRNA decapping in the cytoplasm (Caponigro & Parker, 1995). Similarly, mutations in Rna14p, which is a component of the complex that catalyzes 3' end cleavage and polyadenylation (Minvielle-Sebastia et al., 1994), affect both the formation of 3' ends and the cytoplasmic stability of the ACT1 mRNA (Minvielle-Sebastia et al., 1991). This raises the possibility that Rna14p is involved in specific interactions within the cytoplasm required for maintenance of mRNA stability. Two observations are consistent with this possibility. First, the Rna14p is known to be bound in the cytoplasm (Bonneaud et al., 1994). Second, overexpression of a yeast homolog of the eubacterial and archaeobacterial L1 ribosomal protein partially suppresses the temperature sensitivity of some rna14 alleles (Petitjean et al., 1995). Given all these observations, we propose that the complex of proteins assembled on the 3' UTR during 3' end formation and polyadenylation persists in some form in the cytoplasm and functions to promote the stability of the mRNA in a manner requiring interactions with the translation-termination complex.

MATERIALS AND METHODS

Strains

The strains used in this work were all created by standard genetic methods and are all from an isogenic background based on yRP840 (Hatfield et al., 1996). Specific strain genotypes are as follows:

yRP1209: MATa, leu2, his4, trp1, lys2, ura3, cup1::URA3
 yRP1212: MATa, leu2, his4, trp1, lys2, ura3, cup1::URA3, upf1::URA3
 yRP1211: MATa, leu2, lys2, trp1, his4, ura3, cup1::URA3, dcp1::URA3
 yRP1305: MATa, leu2, lys2, his4, trp1, ura3, cup1::URA3, upf2::NEO
 yRP1306: MAT α , leu2, lys2, his4, trp1, ura3, cup1::URA3, upf3::NEO
 yRP1307: MAT α , leu2, lys2, his4, trp1, ura3, cup1::URA3, xrn1::URA3

Plasmids

Integration vectors to create complete deletions of UPF2 (pRP940) and UPF3 (pRP941) were constructed by PCR to contain 600–800 bases of both 5'- and 3'-flanking sequence around a GPD promoter-neomycin-PGK1-terminator construct (pRP917). The UPF2 deletion removes from 56 bases before the start codon through 124 bases after the stop codon. The UPF3 deletion completely removes from 54 bases 5' of the start codon through 98 bases after the stop codon. The UPF1 disruption with URA3 was obtained from Stuart Peltz and contains the entire UPF1 coding region replaced with the URA3 gene (Leeds et al., 1992). The DCP1, and XRN1 disruptions have been described previously (Larimer & Stevens, 1990; Beelman et al., 1996).

Plasmids containing the CUP1 reporter genes were built in the backbone of p424 (Christianson et al., 1992), a 2 μ TRP1 vector. All of these plasmids contained the GAL1 UAS fused to the PGK1 5' UTR (Heaton et al., 1992) through amino acid 7. This was followed by insertion of a Flag epitope tag in frame and the exact CUP1 coding sequence. A short poly-linker (*Clal*, *Sall*, *PstI*, *SphI*) was placed immediately after the CUP1 stop codon and was fused to the entire PGK1 3' UTR from ~20 nt 3' of the PGK1 translation-termination codon to the *HindIII* site 3' of the PGK1 gene. The sequences from the next base after the CYC1 termination codon extending 1.46 kb 3' were amplified by PCR on genomic DNA, from either the CYC1 wild-type or *cyc1-512* mutant strains, with primers containing *PstI* restriction sites. These PCR products were then inserted into the *PstI* site of the Flag-CUP1 reporter construct, which replaces all of the 3' UTR sequences of PGK1. The plasmid with CYC1 wild-type sequences in this position is pRP947. The plasmid with *cyc1-512* sequences in this position is pRP946. A second set of constructions was done in which the CYC1 and *cyc1-512* sequences were amplified by PCR using primers with *Sall*-*SphI* ends. These products were inserted into the polylinker sequence after the CUP1 stop codon, thus leaving the existing PGK1 3' UTR intact. The wild-type CYC1 plasmid is pRP943 and the mutant *cyc1-512* plasmid is pRP942.

Plasmids carrying the full PGK1 coding region fused to the wild-type CYC1 (pRP945) or mutant *cyc1-512* (pRP944) sequences were built by inserting a 1.2-kb *BglII*-*Clal* fragment containing the 5' coding region of PGK1 from plasmid pRP601 (Muhrad & Parker, 1994) into the CYC1-containing plasmids pRP943 or *cyc1-512* plasmid pRP942.

The nonsense-containing plasmids of PGK1 fused to the CYC1 (pRP956) or *cyc1-512* (pRP952) 3' ends were constructed by replacement of the PGK1-Flag-CUP1 sequences of plasmid pRP942 or pRP943 with a PCR-generated *Apal*-*Clal* fragment. The 3' oligonucleotide used for PCR on an otherwise wild-type PGK1 plasmid (pRP601) contained the nonsense mutation that was located 103 bases into the PGK1 mRNA.

RNA analysis

RNA preparations were done as previously described (Caponigro et al., 1993). Agarose northern gels, containing 10 μ g of total RNA per lane, and subsequent hybridizations were done according to standard procedures. Oligonucleotides used to probe northern are as follows: CUP1 = oRP671;

CYC1 = oRP674; PGK1tag = oRP121. All gels were standardized to loading using the SCR1 transcript (Caponigro et al., 1993).

ACKNOWLEDGMENTS

This work was funded by a grant from the Howard Hughes Medical Institute. The authors thank members of the Parker lab for helpful ideas and comments.

Received April 20, 1999; returned for revision June 2, 1999; revised manuscript received June 30, 1999

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